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Preliminary Assessment of Legacy and Current-Use Pesticides in Franciscana Dolphins from Argentina

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Abstract
The change towards intensive agriculture has led to an increase in the use of pesticides. In addition, legacy pesticides, such as organochlorines are still present in the environment. Ten Franciscana dolphins were accidentally killed by netting in a coastal area of Argentina in Buenos Aires province. From these animals, organochlorine, organophosphate and pyrethroid pesticides were analyzed in liver, bubbler and melon tissues. The concentrations of Σendosulfan ranged from not detectable values (nd) to 3539 ng g^{-1} lw, with the maximum level in melon tissue. DDE was present in 60% of all samples at concentrations from nd to 6672 ng g^{-1} lw, indicating historical DDT contamination. The presence of endosulfan and heptachlor in a nursling calf indicated a transfer of these pesticides through lactational and placental transport. The concentrations of organophosphates and pyrethroids were below the limit of detection, reflecting the low persistence of these compounds.

Keywords Coastal dolphin · Organochlorines · Organophosphates · Pyrethroids · Southwestern Atlantic Ocean · Biomonitor

Pesticides are one of the hazardous groups of chemicals, which are known to interfere with many vital functions of organisms. Although some organochlorine (OCs) pesticides, such as chlordanes, dieldrin and endosulfan, are banned by the UNEP Stockholm Convention, they are still in the environment due to of their high persistence. On the other hand, current use pesticides (CUPs; e.g. chlorpyrifos, diazinon, cypermetrhin, thionazin) are large-scale replacements for globally banned legacy OCs pesticides, but relatively few field studies have investigated the presence of these compounds in the marine environment (Morris et al. 2016; Weber et al. 2010).

Argentina is a farming country and the gradual change towards modern and intensive agricultural activities has led to an increase in the use of pesticides (Pórfido 2014). Estuarine and marine environments are the final receptors of most of these compounds, with an impact on the ecosystem (Arias et al. 2011). Odontoceate cetaceans have certain characteristics that contribute to the accumulation of pesticides in their tissues (top predators, longevity, late maturity, large lipid reserves in fat, high metabolic rates) (Dirtu et al. 2016; Fair et al. 2010). Franciscana dolphin (*Pontoporia blainvillei*) is a small and endemic marine mammal that inhabits the Southwestern Atlantic Ocean. Its geographical distribution ranges from Itaúnas (18°25′S, 30°42′W, Brazil; Siciliano 1994) to Golfo Nuevo (42°35′S, 64°48′W, Argentina; Bastida et al. 2007). The International Union for Conservation of Nature (IUCN) has categorized the species as Vulnerable A3d throughout its geographical range being the most anthropogenically impacted small cetacean (Secchi and Wang 2002). Due to their coastal and estuarine habits, Franciscana dolphins from Argentina inhabit areas with intense human activity which poses several threats to their conservation.

The adverse effects of pesticides in marine mammals is difficult to assess (Dirtu et al. 2016); however, several studies have linked effects on the reproductive (Murphy et al. 2015), endocrine (Hoydal et al. 2016) and immune system (Lehnert et al. 2016), and also with carcinogenesis (Martineau et al. 1994) and skeletal abnormalities (Mortensen

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et al. 1992). Few studies have evaluated pesticides in cetaceans in Argentinean waters (Borrell et al. 1990, 1995; Castello et al. 1997; Durante et al. 2016; Torres et al. 2015), and there are no reports on organophosphates (OPs) and pyrethroid pesticides. Therefore, the aim was to determine the presence of OCs, OPs and pyrethroids pesticides in blubber, melon and liver of *Pontoporia blainvillei* from Argentina.

Materials and Methods

Ten Franciscana dolphins were collected from coastal area of Buenos Aires Province between 2008 and 2011 (Fig. 1). Dolphins were incidentally captured in artisanal fishing nets, being entangled for a period less than 10 h before sampling. The quality of the carcasses was evaluated according to Geraci and Lounsbury (2005), to determine their suitability for the intended study. Total length, weight, and sex were determined for each specimen. Samples of the liver, bubbler and melon were collected and stored at − 80°C until analysis. Age was estimated by Denuncio (2012) using dentine and cementum dental layers to determine growth layer groups (GLGs). Franciscana dolphins were grouped into three age classes: calf, juvenile and adult according to Denuncio 2012.

The extraction and cleaned-up of pesticides in liver was performed by the QuEChERs method described by Anastassiades et al. (2003) with modifications, based on a solid dispersive phase extraction (dSPE). Briefly, a portion of the liver was cut into small pieces (approximately 2 mm) and homogenized until a uniform homogenate was obtained. Approximately 2 g of tissue were weighed into a 50 mL tube, spiked with internal standard *cis*-nonachlor solution (Sigma-Aldrich, Pestanal), and sonicated for 10 min. After this, 15 mL of acetonitrile (ACN)

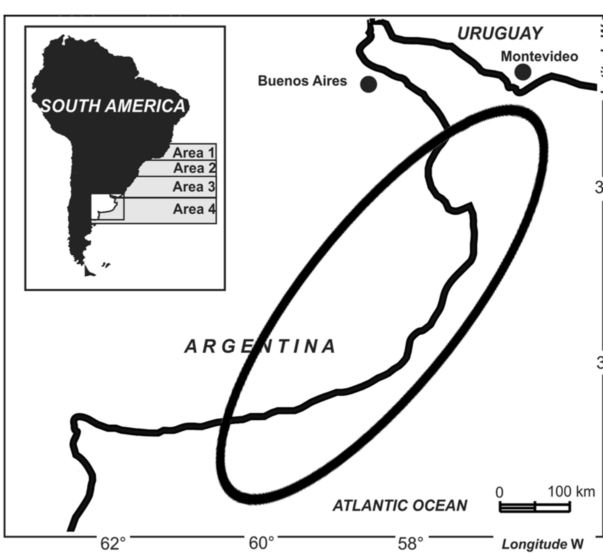

Fig. 1 Study area in Argentinean continental shelf

and 15 mL of hexane were added. The tube was shaken vigorously for 2 min and centrifuged at 5000 rpm. The ACN phase was transferred to a 10 mL beaker and evaporated to 1 mL with gaseous nitrogen. A clean up with previously heat activated florisil (Sigma-Aldrich, Pestanal), PSA y C18 was performed and the supernatant was concentrated under a nitrogen flow until near dryness and re-dissolved in 200 µL of acetone.

QuEChERs technique was developed for samples with a high water content. Due to the high lipid content in blubber and melon, it was not possible to apply the method described above. Therefore, approximately 1 g of tissue was weighed and extracted with sulfur ether in an automated hot Soxhlet extractor for 5 h, and the lipid content was determined gravimetrically. The clean-up was performed in a column (C18, Enviro Clean Extraction Columns), previously activated with ACN. The cleaned eluates were concentrated under a nitrogen flow until near dryness and re-dissolved in 200 µL of acetone. Detection and quantification of compounds in liver, bubbler and melon extracts were performed by Gas Chromatography-Mass Spectrometry (GC-MS, Agilent 7890).

For quality control/quality assurance (QC/QA) purposes, samples were fortified with 0.05 mg kg^{-1} of the analytical standard *cis*-nonachlor solution (Sigma-Aldrich, Pestanal), and the recovery were between 93.0 and 101.5%. In addition, blanks of solution and matrix were checked in each batch of procedure; both were below the detection limits for all pesticides analyzed. The regression values of the standard curves for the compounds were > 0.999 (95% of confidence). The slope and adjustment were checked by the Significance for Linear Regression and Lack of Fit tests, respectively ($p < 0.01$). The precision was checked through the repeatability in batch of 10 determinations, and the %RSD obtained was < 6.5 for all pesticides. The limit of detection achieved for OCs was 10 ng g^{-1}, and for OPs and pyrethroids was 30 ng g^{-1}. Concentrations lower than these values were considered as not detectable (nd). The results were expressed in ng g^{-1} lw

In order to perform statistical analysis, a value equal to half of the detection limit was arbitrarily assigned for those samples that showed values below this limit. Normality was tested using the Kolmogorov–Smirnov test, whereas homoscedasticity with Levene test. Statistical differences were verified using non-parametric tests (Mann Whitney and Kruskal Wallis tests). All analysis were performed with the STATISTICA® 6.0 program (Statsoft, Inc.).

Results and Discussion

All carcasses were in good condition (Code 2; Geraci and Lounsbury 2005), indicated by the presence of rigor mortis. Biometric measurements of the specimens and the concentrations of detectable pesticides are shown in Table 1.

The following pesticides showed concentrations below the detection limit in all samples: aldrin, *cis*-chlordane, *trans*-chlordane, p,p′-DDT, dieldrin, endrin, heptachlor epoxy, hexachlorobenzene, *cis*-nonaclor, *trans*-nonaclor, acephate, azinphos methyl, chlorpyrifos ethyl, chlorpyrifos methyl, diazinon, dichlofenthion, dichlorvos, dimethoate, disulfoton, fenitrothion, phonofos, malathion, metamidofos, methidathion, monocrotophos, parathion ethyl, parathion methyl, pirimiphos methyl, thionazin, cypermethrin, deltamethrin, fenvalerate, permethrin.

The concentrations of Σendosulfan (corresponding to endosulfan I – EI-, endosulfan II – EII- and endosulfan sulfate -ES-) presented range values of nd–275.97 ng g^{-1} lw in blubber, nd–3539.57 ng g^{-1} lw in melon, and nd–88,341.03 ng g^{-1} lw in liver. Endosulfan compounds were found in at least one of the tissues in all Franciscana dolphins; and its presence was related to a wide use of the pesticide in Argentina and recent prohibition in 2013. Information related to endosulfan and its metabolites in cetaceans is limited. In *P. blainvillei* there is only one report of blubber concentrations of EII in Brazil, and they were below the limit of detection (Leonel et al. 2010). Besides, the blubber concentrations of EI and EII in Franciscana dolphin were higher than those reported for bottlenose whale – *Hyperoodon ampullatus* – , bottlenose dolphin – *Tursiops truncatus* – , striped dolphin – *Stenella coeruleoalba* – and white-sided dolphin – *Lagenorhynchus acutus* – (Table 2).

p,p′-DDE metabolite was present as a unique representative of the DDT group in 60% of all samples, p,p′-DDD was present only in one specimen (Table 1), and no DDT was detected. The concentrations of DDE found in Franciscana dolphin were within the range reported for the species and other cetaceans (Table 2). In Argentina, DDT was banned in 1992, and the predominance of the metabolite with respect to the original compound indicates a not recent entry of the pesticide into the environment.

Heptachlor was found only in 30% of dolphins, with values much higher than those reported for other cetaceans

Table 1 Biological information and concentrations of pesticides (ng g^{-1} lw) in the blubber (B), melon (M) and liver (L) of Franciscana dolphins Diet information was obtained from Denuncio (2012)

Code	Sex	Age class	Estimated age (years)	Diet	Total length (cm)	Weight (kg)	Tissue	Pesticide	Concentration
D1	F	Adult	6.7	Solid	140	29.0	B	ES	25.5
							L	ES	1973.6
							B	DDE	267.6
D2	M	Juvenile	2.9	Solid	115	22.1	B	DDE	22.1
							B	Heptachlor	64.2
							M	EI	363.4
							M	EII	545.1
D3	F	Calf	< 1	Milk	74	4.1	B	EII	131.8
							L	ES	8773.6
							B	Heptachlor	70.3
D4	M	Calf	< 1	Mixed	84	9.7	L	ES	6578.6
D5	M	Juvenile	1.2	Solid	91	11.5	B	ES	89.5
							B	DDE	246.1
							M	DDE	61.3
							M	DDD	735.0
D6	F	Adult	10.5	Solid	139	18.6	M	DDE	178.4
							L	DDE	6672.6
							L	EII	2067.6
							L	ES	8928.1
D7	F	Juvenil	2.7	Solid	109	na	B	DDE	24.9
							M	EII	230.0
							L	EI	88341.0
D8	F	Adult	4	Solid	136	31.1	L	ES	12217.4
							L	Heptachlor	6484.6
D9	F	Adult	7	Solid	126	22.5	M	EII	1461.1
D10	M	Juvenile	2.3	Solid	119	16.5	B	EII	29.2
							L	DDE	1879.6

M male, *F* female

Table 2 Concentrations of pesticides (ng g^{-1} lw) reported in other cetacean species

Specie	T	Compound					
		DDE	Endosulfan I	Endosulfan II	Endosulfan sulfate	Heptaclhor	Reference
P. blainvillei	B			nd		nd	Leonel et al. (2010)
	B	M: 0.79 ± 0.71 F: 0.75 ± 0.40					Castello et al. (1997)
	B	M: 1.97 ± 1.27 F: 0.3 ± 0.18					Borrell et al. (1990, 1995, 1997)
Tursiops truncatus	B	1.4–22.72	0.85–1.11	2.06–27.39	3.05–37.29	1.11–12.57	Delgado-Estrella et al. (2015)
	B	M: 188–14,300 F: 5500–56,300	M: 0.4–4.1 F: 0.5–5	nd	M: 1.1–2.6 F: 1.1–5.1		Fair et al. (2010)
	B	M: 9952–80,252 F: 1163–15,042		nd	nd		Hansen et al. (2004)
	L	0.041–9.78					Shoham-Frider et al. (2009)
	B	0.71–135					
Hyperoodon ampullatus	B	M: 8218 ± 5846 F: 1887 ± 506	M: 0.9 ± 2.3 F: 15.8 ± 28.7	M: 73.2 ± 78.3	M: 28.3 ± 25.8 F: 27.7 ± 18.7	M: 1.6 ± 3.7 F: 1.1 ± 1.3	Hooker et al. (2008)
Lagenorhynchus acutus[a]	B		0.01–0.33				Tuerk et al. (2005)
Stenella coeruleoalba	B		4.6–134			1.6–17	Wafo et al. (2012)

Data are presented as ranges or as mean ± SD

nd not detectable, *T* tissue, *B* blubber, *L* liver, *M* male, *F* female

[a]Concentration expressed in ng g^{-1} ww

(Table 1). Its metabolite heptachlor epoxide was not detected, although it is considered the main compound found in marine mammals (Dierauf and Gulland 2001). This pesticide was widely used in Argentina as an insecticide in potato crops (Miglioranza et al. 2003), and although its use was banned in 1992, the presence of the parent compound in *P. blainvillei* tissues indicates a recent contribution of the contaminant to the environment.

The D3 dolphin, a nursing calf, presented endosulfan compounds and heptachlor in its tissues, indicating a transference of the pesticide through milk and/or previous placental exposure. The concentrations of these pesticides were similar to adult Franciscana dolphins. The mother-calf transfer of organochlorine pesticides has been reported for other dolphin species (Stockin et al. 2007).

The OPs and pyrethroid pesticides analyzed were found below the limit of detection in Franciscana dolphins. Information about the presence of these contaminants in cetaceans is scarce, due to the low environmental stability and persistence of these compounds compared to OCs. Hernández et al. (2000) reported non-detectable levels of OPs in the fin whale (*Balaenoptera physalus*), which is in accordance with the results in the Franciscana dolphin. By other way, Alonso et al. (2012, 2015) reported the presence of 12 pyrethroids in tissues of the species in Brazilian waters, although several samples presented levels below the detection limit. Transplacental and lactational transfer was also found. The authors proposed that the compounds are detected in the first moments of life, during pregnancy and lactation. Although when dolphins reach sexual maturity the pyrethroids would begin to be metabolized, and consequently the decrease or disappearance in the organism would be evident.

The presence of organochlorine pesticides in Franciscana dolphin is related to their persistence in the environment and low metabolic degradation. The concentrations of organophosphates and pyrethroids below the limit of detection reflected the low persistence of these compounds in the environment. In addition, the species could be considered as a good biomonitor of pesticides in Argentinean waters. On the other hand, it is necessary to carry out more detailed in-time studies of the profiles of CUPs in Franciscana dolphin, in order to reach a better understanding of the possible biological effects and the risks for the species in the present.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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